N73-17111

QUANTITATION OF BURIED CONTAMINATION BY USE OF SOLVENTS

Semi-Annual Report

for

July 1, 1972 through December 31, 1972

Supported by

NASA Grant NGR 35-001-012
Supplement No. 2

Submitted to the

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

by the

Department of Polymers and Coatings
North Dakota State University
Fargo, North Dakota 58102

Investigators: S. Peter Pappas, Paul Hsiao, and Loren W. Hill

INTRODUCTION

The objectives of the overall project are: (1) to develop non-sporicidal methods for solvent degradation of cured polymeric resins that are used in spacecraft and (2) to determine whether reaction conditions during resin cure cause decontamination of the component that is being fabricated. The previous reports[1,2] described the application of solubility parameter methods to the degradation of amine cured epoxy resins, and the solubilization of cured silicone resins in amine solvents. Spore viability results on exposure to amine solvents as well as to a silicone resin (DC840) dissolved in amine solvents have also been reported. The present report describes (1) continued investigation of the sporicidal properties of amine solvents that solubilize silicone resins, (2) recovery studies on a silicone potting compound (RTV 41) that is used in spacecraft, and (3) confirmation that spores remain viable during chemical cure of this potting compound.

In the preceding semi-annual report[2] it was noted that spore recoveries following amine treatment were reduced by a sporestatic effect of residual amine following dilutions of 10^3 or 10^4 prior to plate counting. This effect is negligible at dilutions of 10^5 or 10^6. Greater dilution is required with the spore suspension currently in use because the spore concentration is higher (about 10^8/ml in ethanol). The original spore sample was suspended in water, and the heat of mixing of water and amine also contributed to lower recoveries. With

the ethanol suspension, the heat of mixing is much lower, and as a result higher recoveries are observed.

Extension of the viability study to a chemically cured silicone potting compound (RTV 41) required the use of another solvent for dilution. The cured silicone rubber was dissolved in butylamine, and recovery was determined by the plate counting technique with series dilution in benzene. Of course, it was not possible to dilute the butylamine-silicone solution with water because the silicone would precipitate on addition of water. Benzene was selected because it has been reported to be less toxic[3] to spores than many other organic solvents and because it is a good solvent for the silicone. The use of benzene for series dilution led to a study of spore viability in benzene, and to control experiments involving addition of spore suspension to butylamine followed by series dilution in benzene with no silicone rubber or curing agent present.

EXPERIMENTAL

Procedure

An inoculated silicone rubber potting compound was prepared from the following:

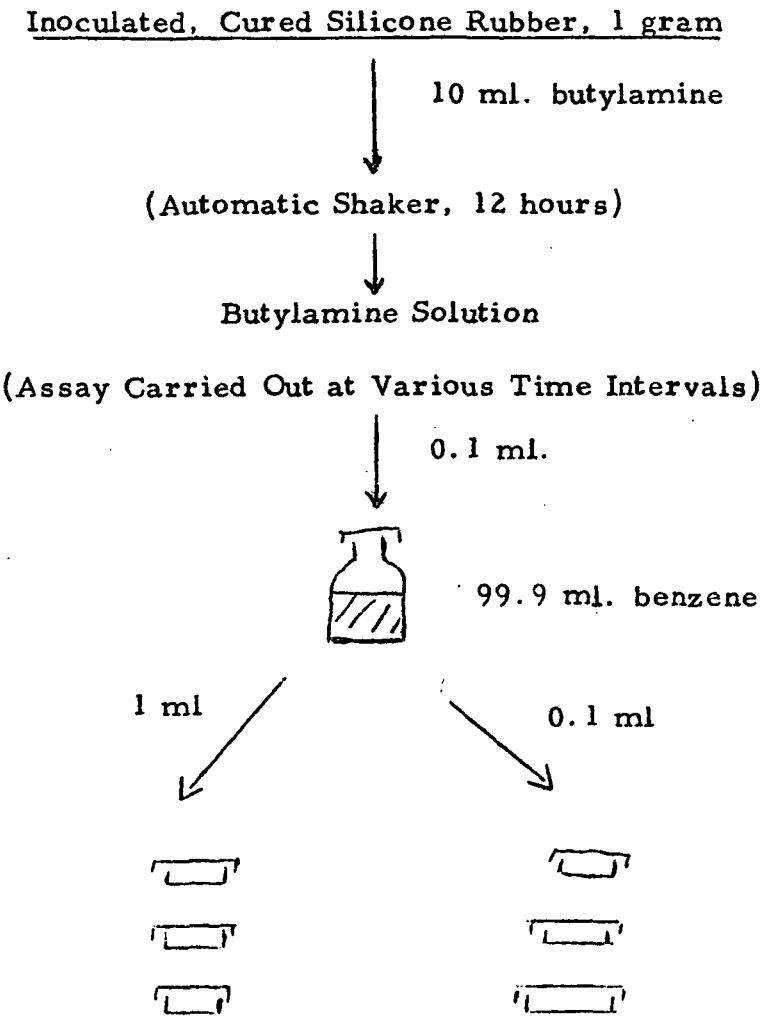
(a) Silicone prepolymer, RTV 41 - 1.0 gram

(b) Curing agent (dibutyl tin dilaurate) - 1 drop

(c) Spore suspension (10^8/ml. in 95% ethanol) - 0.10 ml.

The samples were prepared by charging (a) and (c) to a 25 ml. round bottom flask and immediately adding the curing agent (b). The ratio

of (a) to (b) corresponds to the supplier's recommendations. After 24 hours curing time, 10 ml. of butylamine was added, and solubilization was assisted by placing the sample on an automatic shaker. The sample was completely dissolved in 12 hours at room temperature. The assay was carried out as indicated below:

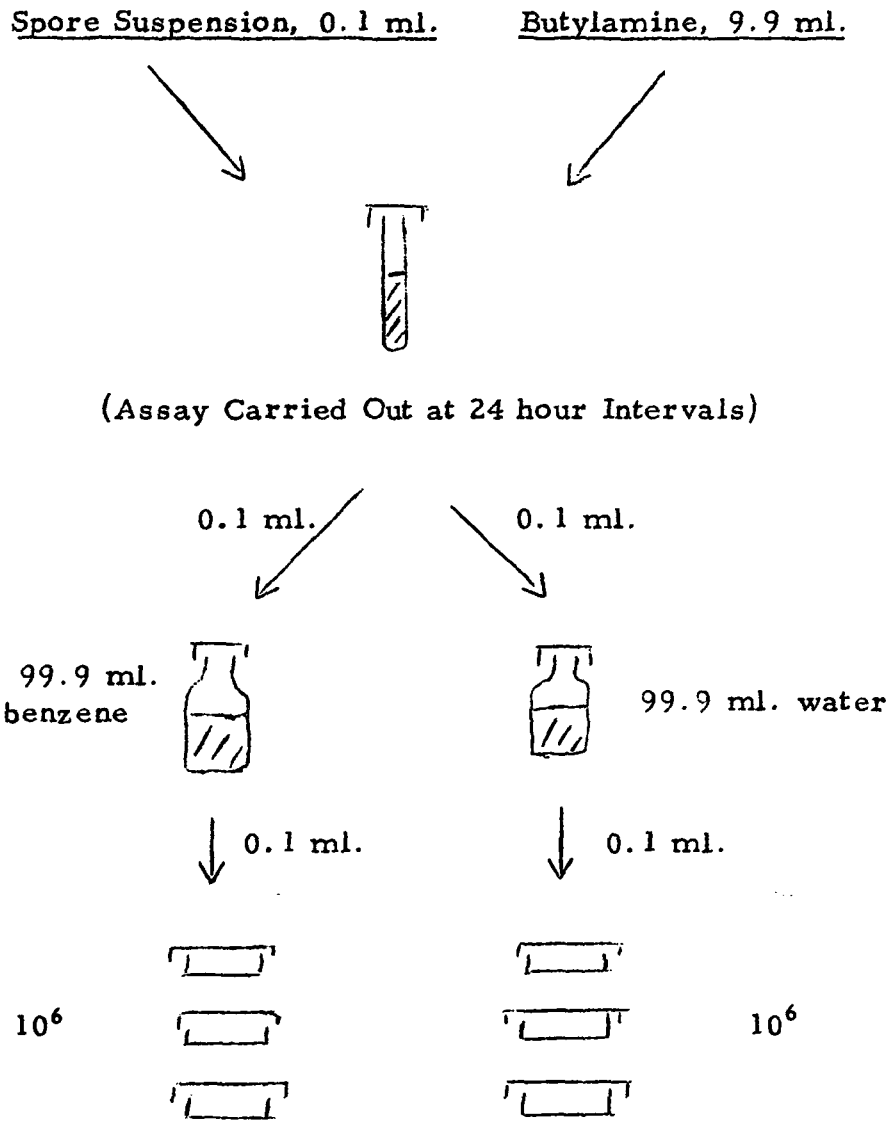


48 hour incubation at 32 °C before colony counting

The dilution factor including the inoculation step is 10^5 or 10^6; between 30 and 300 colonies were observed per plate at 10^6 dilution. Assays were done in triplicate with the results indicated in Table I.

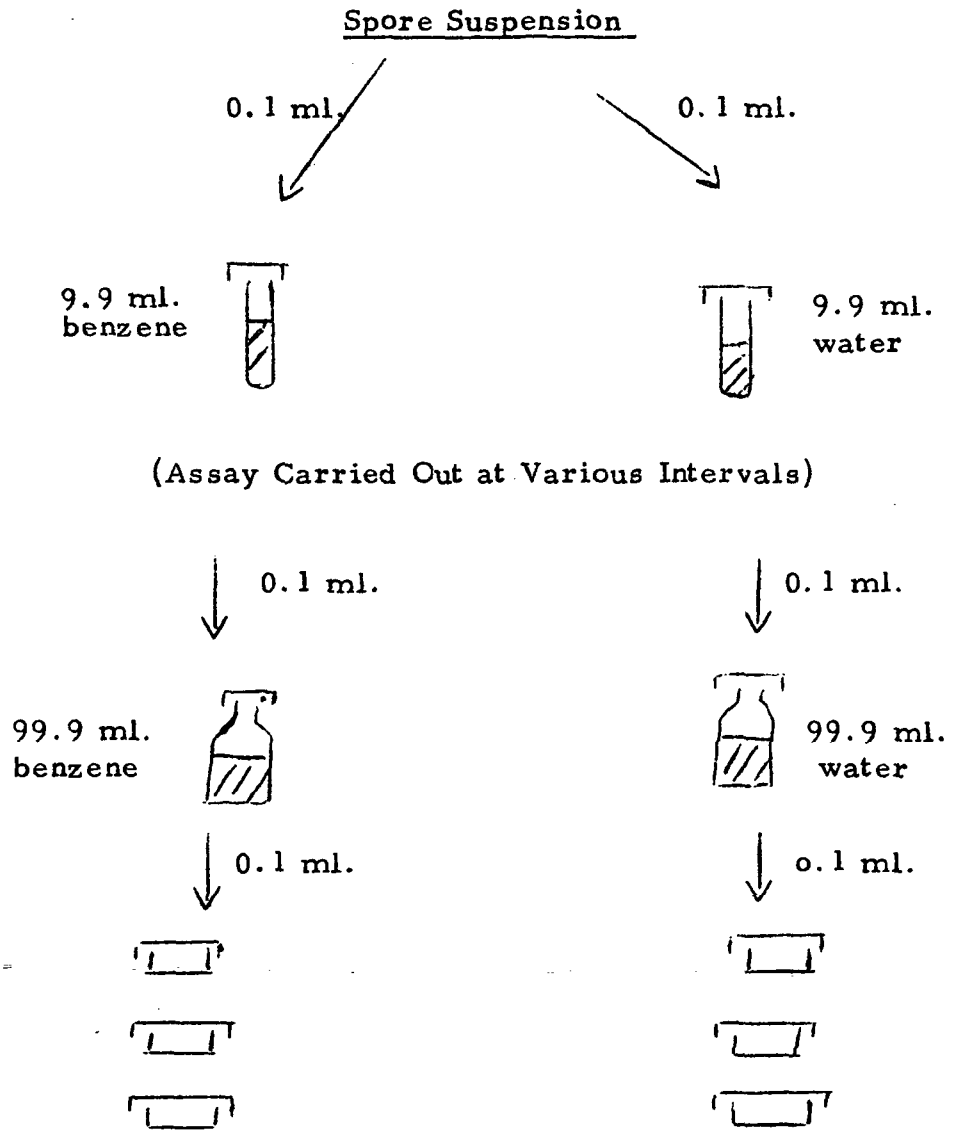
Controls in which the same procedure was followed except that inoculation was omitted, were all negative, i.e. no colonies were observed.

In the positive control for the experiments described above, silicone prepolymer and curing agent were omitted, but the amine solvent was included. Water was also used as a diluent for comparison with the results obtained using benzene.



The results for this positive control are given in Table II.

The high recoveries observed with the butylamine-benzene system in comparison with the butylamine-water system were unexpected, and therefore a comparison of benzene dilution and water dilution was carried out with butylamine omitted. The results are presented in Table III.



48 hour incubation at 32 °C before colony counting

Materials

1. Butylamine - Eastman Organic Chemicals reagent grade distilled.

2. Spore Suspension - A Bacillus subtilis var. niger ... ca. ca. 10^8/ml in 95% ethanol-Produced in SSM-10 liquid sporulation medium. Designation "BGSSM-10". Obtained from Dr. Walter W. Bond, Experimental Microbiology Unit, Environmental Microbiology Section, US DPHA, DHEW, Phoenix, Arizona.

3. Liquid Silicone Rubber, RTV-41 (Lot No. BE 180) - General Electric, Silicon Products Department, Waterford, New York.

4. RTV Silicone Rubber Curing Catalyst - Dibutyl Tin Dilaurate - General Electric.

5. Benzene - Baker Chem. Co., reagent grade, Lot No. 9154.

6. Tryptic Soy Agar (dehydrated) Control 577932 - Difco Laboratories, Detroit, Michigan

RESULTS AND DISCUSSION

Colony counts obtained from inoculated silicone rubber samples are presented in Table I. Recoveries are very high indicating that neither the silicone prepolymer nor curing agent are sporicidal. Furthermore, it can be concluded that the curing process is not sufficiently exothermic to reduce spore viability. Of course, the small size of the sample would tend to minimize temperature increase. Comparison with the control in which silicone rubber is omitted (Table II) shows that there is essentially no reduction in viability by the silicone rubber components or curing reaction.

A comparison between colony counts observed with benzene

dilution and with water dilution following various periods of time in butylamine is given in Table II. There is no significant reduction in colony counts with benzene dilution; however, when water is used as a diluent colony counts decrease as time in butylamine increases. Colony counts obtained with water dilution of the ethanol suspension in the absence of butylamine are given in Table III. On the basis of a limited amount of data, the spore population appears to decrease more rapidly with time in water than in butylamine, but the initial counts are lower following butylamine treatment. Investigation of the change in colony count with time is continuing. This decrease with time is not understood. A possible explanation is that some of the spores germinate, but then the organism dies prior to plate counting due to lack of nutrients.

The colony counts obtained on adding spore suspension to benzene in the absence of butylamine are very erratic as indicated in Table III. The scatter is too great to draw firm conclusions. It is difficult to understand why treatment of spores with butylamine followed by benzene dilution (Table II) should yield higher colony counts than treatment of spores with benzene followed by benzene dilution (Table III).

One of the most surprising observations is that the spore population of the ethanol suspension is calculated to be 1.6×10^8/ml. from water dilution runs while values of about 2.5 to 10^8 to 2.8×10^8/ml. are obtained from the system containing silicone rubber, butylamine,

and benzene (Table I) or from the system containing butylamine and benzene (Table II). If the spore population were based on our water dilution results, recoveries exceeding 100 % would be indicated. The remarkable viability of spores in the butylamine-benzene system has been demonstrated repeatedly in our laboratory, but since the result is unexpected, we are making arrangements to have the experiments repeated at another laboratory.

CONCLUSIONS

It has been established that:

(1) the recovery of spores from a cured silicone potting compound (RTV 41) is very high (>90 %) when the silicone rubber is dissolved in butylamine and series dilution is carried out with benzene prior to plate counting, and

(2) spores remain viable during chemical curing of RTV 41 silicone potting compound in a 1 gram sample.

TABLE I

Table I. Colony Counts Obtained from Butylamine Solution of Inoculated Silicone Potting Compound, RTV 41, Using Series Dilution in Benzene; Dilution Factor = 10^6

Experiment Number	Time*	Number of Colonies			Average
I	2 days	262	290	270	276
	3 days	286	269	256	271
	4 days	268	228	251	249
	7 days	288	285	234	269
	11 days	245	280	213	246
	16 days	228	233	236	232
	17 days	281	219	207	236
	20 days	262	232	246	247
	22 days	258	245	250	251
	25	237	228	231	232
	48 days	226	202	182	203
II	1.5 days	304	254	260	270
	3 days	268	272	273	271
	7 days	269	251	259	260
	11 days	274	263	262	266
	20 days	248	274	246	256

* Zero time is taken as the instant of addition of butylamine to the inoculated, cured silicone rubber. Samples kept at room temperature.

TABLE II

Table II. Colony Counts Obtained After Adding Spore Suspension (0.1 ml.) to Butylamine (9.9 ml.) in Absence Silicone Potting Compound Using Series Dilution in Benzene and in Water; Dilution Factor = 10^6 in Both Cases.

Time after mixing* (days)	Number of Colonies					
	Benzene as diluent			Water as diluent		
			(average)			(average)
0	224	251	(238)	102	103	(102)
1	278	289	(283)	101	104	(102)
2	263	282	(272)	97	98	(97)
5	202	206	(204)	82	69	(75)
6	234	244	(239)	13	9	(11)
9	263	212	(238)	14	9	(12)
11	261	238	(249)	15	11	(13)
14	282	264	(273)	12	5	(8)
15	271	272	(271)	10	8	(9)
28	194	194 206	(198)	18 10	9	(16)

* Samples kept at room temperature.

TABLE III

Table III. Colony Counts Obtained After Adding Spore Suspension (0.1 ml.) to Benzene (9.9 ml.) Followed by Series Dilution in Benzene and to Water (9.9 ml.) Followed by Series Dilution in Water; Dilution Factor = 10^6 in Both Cases.

Experiment Number	Time after mixing (days)*	Number of Colonies						
		Benzene as diluent				Water as diluent		
					average			average
1	0	6	22	13	(14)	169	161	(165)
	4	44	37	10	(30)			
2	0	10	5		(7)	148	158	(153)
	1.5	3.0	3.5		(3.2)			
3	0					151	154	(153)
4	0	9	6	5	(7)	141	144	(143)
	5 hrs	56	73		(65)			
	36 hrs	18	12		(15)			
5	0	2	0	11	(4)	157	173	(165)
	1	40	4		(22)	102	109	(105)
	2	1	6		(4)	22	22	(22)
	3	0	2		(1)	12	11	(12)
	4	24	3		(13)	10	15	(12)
6	0	0	3		(2)	158**	196	(177)
	1	32	45		(36)	109	108	(109)
	2	5	1		(3)	33	22	(28)
	3	26	26		(26)	13	9	(11)
	4	27	3		(15)	39	24	(32)

* Samples kept at room temperature.

** 0.1 ml. spore suspension in 9.9 ml. distilled water was kept in refrigerator.

REFERENCES

1. A. E. Rheineck and R. A. Heskin, "Quantitation of Buried Contamination by the Use of Solvents", First Interim Report, Part I, NASA Grant No. NGR-001-012, February, 1972.

2. S. P. Pappas, P. Hsiao, and L. W. Hill, "Quantitation of Buried Contaminants by the Use of Solvents", Semi-Annual Report (1/1/72 - 6/31/72), NASA Grant No. NGR-001-012, July, 1972.

3. "Microbial Cell Recovery from Solid Materials", Final Summary Report, NASA Contract No. 950740, The Jet Propulsion Laboratory, Pasadena, Calif., p. 88, May, 1968.